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Scientific Article

IN VITRO CULTURE OF *Amorphophallus titanum* (Becc.) Becc. ex Archang AT BOGOR BOTANIC GARDENS

Kultur In Vitro *Amorphophallus titanum* (Becc.) Becc. ex Arcang di Kebun Raya Bogor

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Abstract

In vitro culture of *Amorphophallus titanum* (Becc.) Becc. ex Arcang began more than a decade ago at Bogor Botanic Gardens. This paper is a summary of the last three years activities on the propagation of *A. titanum.* The initial cultures originated from different sources some of them were from the gardens collections. Different parts of the plant were used as explants; axillary buds of the corm, leaf veins and cataphyll. Murashige and Skoog (MS) formulation was used as the basal media; and different plant growth regulators or organic substances were added to find out the most suitable media for inoculation and for development of callus, root and shoot. Different types of cultures were developed and single shoots or multiple shoots as well as compact and friable callus were obtained from different explant sources. Zeatin added to MS medium gave the best result for shoot and root induction and the addition of banana showed promising results for shoot maturation in culture. The overall trials are described in this paper.

Keywords: *Amorphophallus titanum*, Bogor Botanic Gardens, *in vitro*, propagation

Abstrak

Kultur *in vitro Amorphophallus titanum* (Becc.) Becc. ex Arcang dimulai lebih dari satu dasawarsa yang lalu di Kebun Raya Bogor. Makalah ini merupakan ringkasan kegiatan perbanyakan *A. titanum* tiga tahun terakhir. Sumber kultur berasal dari berbagai sumber; beberapa berasal dari koleksi kebun. Bagian-bagian yang berbeda dari tanaman asal digunakan sebagai eksplan: mata tunas samping umbi, tulang daun dan helai katapilnya. Medium dasarnya menggunakan formula Murashige & Skoog (MS); dan berbagai zat pengatur tumbuh atau bahan-bahan organik ditambahkan untuk mendapatkan medium yang paling sesuai untuk inokulasi dan pertumbuhan kalus, akar dan tunas. Berbagai bentuk kultur, tunas tunggal maupun ganda juga kalus yang padat dan remah tumbuh dari sumber eksplan yang berbeda. Zeatin yang ditambahkan ke medium MS memberikan hasil terbaik untuk induksi tunas maupun akar dan penambahan pisang menunjukkan hasil yang lebih baik untuk pendewasaan tunas di dalam kultur yang dicoba. Berbagai percobaan yang dilakukan dituliskan dalam makalah ini.

Kata kunci: *Amorphophallus titanum*, *in vitro,* Kebun Raya Bogor, perbanyakan

INTRODUCTION

Amorphophallus titanum (Becc.) Becc. ex Arcang *is* a charismatic plant. This perennial herb with solitary inflorescence always attracts visitors when its unusual inflorescence blooms in Botanic Gardens. Although this species is native to Sumatra, not all Indonesians have had the opportunity to see the beauty of the inflorescence or its unusual characters. In order to introduce *A. titanum* to other places, it should be propagated. Historical review and observations of this species show that it varies in size, colour of the spadix and spathe, flowering period as well as leaf characteristics (Gandawijaja *et al.*, 1983); vegetative propagation is a suitable way to maintain this variability.

Previous works by Asokan *et al.* (1984) produced leaflets from the corm callus of *A. rivierei,* and later the lateral buds of the cormel of *A. campanulatus* var. *hortensis* (now *Amorphophallus paeoniifolius*) grew into callus and more plantlets were found on Murashige & Skoog medium with the addition of 5 µM (1 ppm) NAA and 0.05 µM (0.01 ppm Kinetin) (Irawati *et al.*, 1986). *A. titanum* and *A. rivierei* mid-vein explants of leaflet were first tried by Kohlenbach and Becht (1988) with positive results; firstly, with the formation of green calluses, and later the development of rooted plants, and finally the formation of corms in the greenhouse.

Vegetative propagation is also possible using cubes cut from the corm, which are able to grow into plantlets on sand, although it was noted that the wounded tuber is very susceptible to bacteria and fungi (Prana, 1970). Later, propagations of another *Amorphophallus* species, *A. muelleri,* were conducted using the shoot tip (Imelda *et al.*, 2007) and petiole (Imelda *et al.*, 2008).

An embryoless seed portion of *A. titanum* produced a shoot and roots after being treated with Benzyl aminopurine solution (Prana, 2000). Prana also mentioned the successful attempt to propagate *A. titanum* using an *in vitro* technique by Imelda (unpublished) in the Research Center for Biotechnology, Cibinong.

Research on in vitro culture of *A. titanum* was initiated in December 1991 at the Treub laboratory. Propagation of *A. titanum* through *in vitro* culture was then continued by Irawati (2011) and Witjaksono *et al.* (2012) using different parts of the plants as explants.

Trans-cinnamic acid (tCA) is an anti-auxin which is rarely added to the plant culture medium. However, Kim & Kim (1998), reported that tCA and BA or BA alone were efficient to break the dormancy of the vegetative buds of ginger. Similar results were obtained by Ball *et al.* (1974-1975) by adding tCA to Knop medium to release the apical dominance of *Phalaenopsis* flower stalk.

Compton and Mize (1999) noted that repeat experiments must be conducted over time in order to validate the result of previous studies, since physiological differences among stock plants from which explants are obtained are inevitable.

Genetic variations of 25 accession numbers of *A. titanum* were studied by Poerba and Yuzammi (2008). The results indicated that genetic variation among genotypes were more diverse than genetic variation among populations.

To maintain the variability of different genotypes, vegetative propagation is important, such as through *in vitro* culture.

Efforts to propagate this species were conducted using different parts of the plant. The cataphyll of *A. titanum* have never been described they have different sizes and cover the young leaf, while the sheath of the inflorescence is conspicuous as the inflorescence is the most attractive part of the plant. Ideally, the material used for propagation should not cause damage to this precious plant therefore the leaf-vein and leaf sheath were chosen as explants.

The inoculation technique to obtain sterile explants is an important part of in vitro culture. The success of the culture is greatly

influenced by the nature of the culture medium used. Different plant growth regulators have been tested at different concentrations to induce embryogenic callus. Growth and morphogenesis in vitro are regulated by the interaction between endogenous growth substances and the growth regulators supplied in the medium.

MATERIALS AND METHODS

Plant Materials

The explants originated from: a) lateral buds from the corm (when the shoot tip of the newly collected specimen rotted); b) young leaf-vein from the field and from the Bogor Botanic Garden collections) the cataphyll from the Cibodas Botanic Garden collections (Fig. 1).

Medium

The basal medium was Murashige & Skoog (MS). Plant growth regulators tested were: 2,4-D, BA, GA_3, Kinetin, 2-iP and Zeatin all with NAA added to the medium. Organic materials added to the media were: banana, coconut water and activated charcoal. Trans-Cinnamic Acid (tCA) as an anti-auxin was also employed at different concentrations in combination with light/dark conditions.

Methods

Sterilization: Washing the source plant materral in running water, followed by surface sterilization with NaClO used gradually from 0.525% to 0,2625% repeated twice for 10-15 minutes each, with 20% Streptomycin sulphate and with partial vacuum during sterilization to give better results.

Histological studies were conducted to observe the origin of shoot or root in different types of callus with standard preparation. Some shoots during subculture were cut into pieces and planted to the fresh medium then inoculated as explants.

When the plantlet has at least one leaf and roots, it is ready to be acclimatized in the shadehouse. The plantlet was first soaked in lime solution to prevent rotting, then was planted on sand, soil mixture, bamboo compost or a mixture of cocopeat, shredded tree fern root and husk charcoal. The plantlet was covered with a plastic bag to keep enough humidity. When the roots were established, soil mixture was added to prevent drying, and the plastic cover was removed.

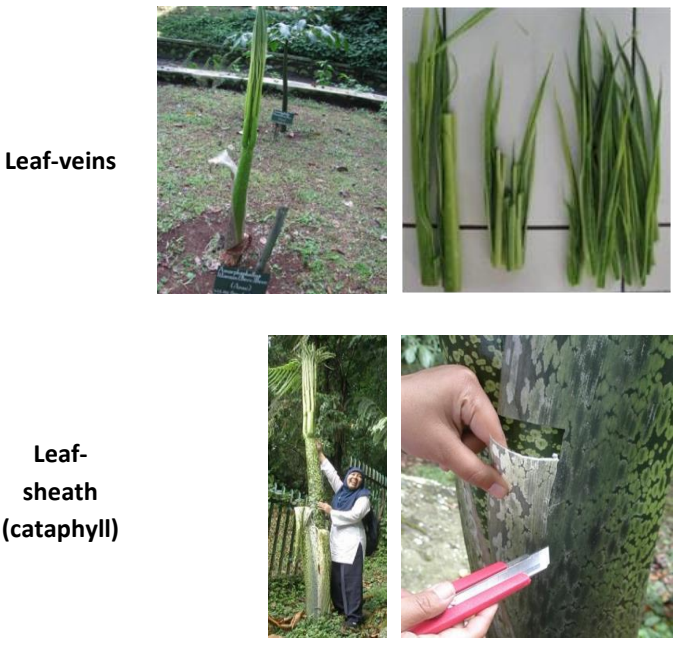

Figure 1. Leaf-vein (top) and leaf-sheath (bottom)

RESULTS AND DISCUSSIONS

Sterilization

Explants from young materials are relatively easy to disinfect compare to the old parts of the plant, because young materials only in a shorter period exposed to the possible unsterile environment and partial vacuum during sterilization gave better results.

Explant Inoculation

The inoculation medium that is suitable for leaf-vein and leaf sheath development is MS + 1 mg/l NAA + 1 mg/l BA. In that media the explants swell and grow into solid callus (Fig. 2).

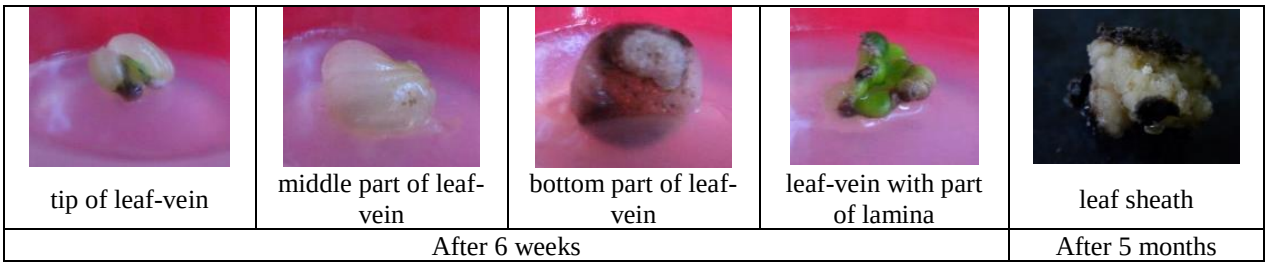

Figure 2. Explants of leaf-vein after 6 weeks and explants of leaf-sheath after 5 months in culture.

All explants (lateral bud, leaf-vein and leaf sheath) grew into solid and friable callus, small shoot and roots. Hyperhydricity was observed in many cultures, and should be prevented in the future. Hyperhydricity is caused by the stressing condition of the *in vitro* plant cultures such as: high relative humidity, accumulation of gases, high osmoticity of the medium. Hyperhydricity often irreversible and the culture easily breakable also unable to develop normaly (Kevers *et al.,* 2004). Different types of callus with different colours were observed as shown in Fig. 3.

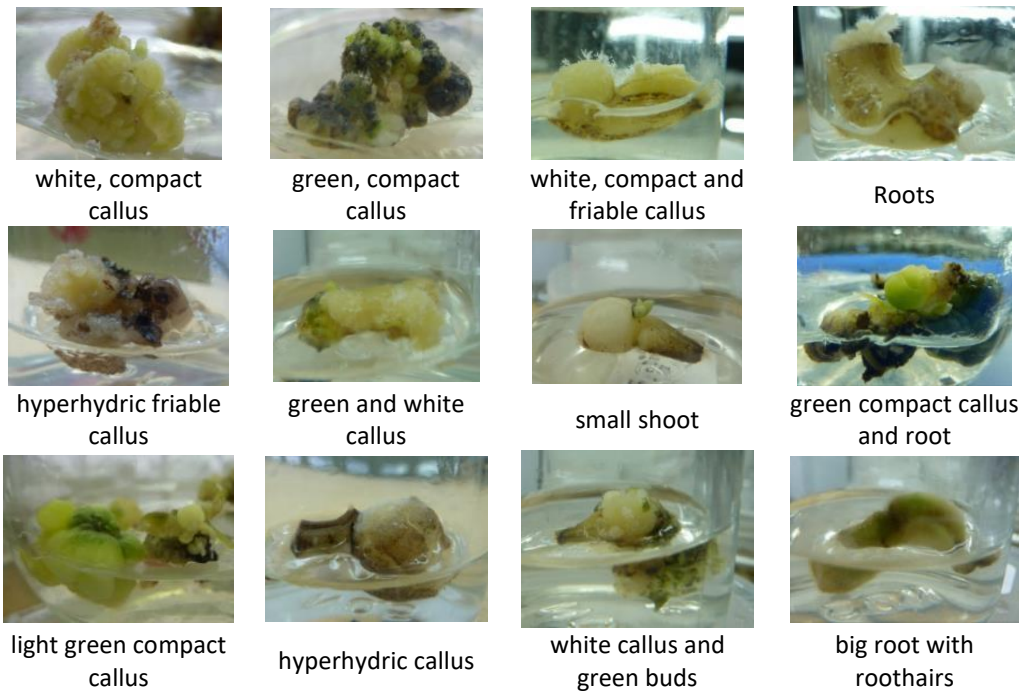

Figure 3. Different types of *Amorphophallus titanum* calli in culture.

At the end of the experimental series, the total number of cultures are shown in Fig. 4. Since 2012, research on this species has continued, as seen in Table 1.

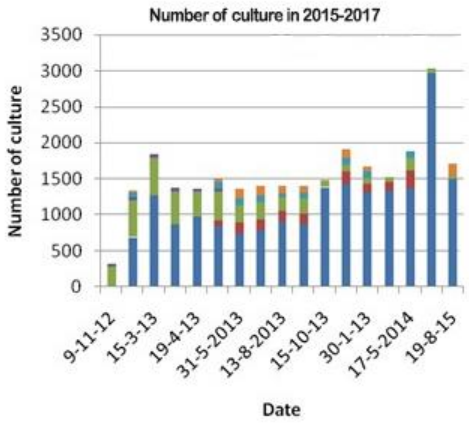

Figure 4. Total number of *Amorphophallus titanum* cultures in Bogor Botanic Gardens, 2012-2015.

Table 1. Effect of some plant growth regulators and other complex additives on *Amorphophallus titanum* cultures.

No.	Substances added to MS media	Amount per liter	NAA per liter	Result
1	2,4-Dichlorophenoxy acetic acid (2,4-D)	1 mg	1mg	Compact and/or friable callus, yellowish green, dark green, roots (57%) and shoot (21%)
2	Benzyladenine (BA)	0.1 mg; 1 mg	1mg	Compact and/or friable callus, some with roots for 0.1 mg BA; Compact and/or friable callus, small shoots, shoots, few with roots for 1.0 mg/l BA
3	Gibberellic acid (GA_3)	1 mg	1mg	Compact and/or friable callus, small shoots, shoots, few with roots
4	6-Furfurylaminopurine (Kinetin)	1 mg	1mg	Compact and/or friable callus, small shoots, shoots, few with roots
5	6-(γ,γ-Dimethylallylaminopurine (2-iP)	1 mg	1mg	Compact and/or friable callus, small shoots, shoots, few with roots
6	4-hydroxy-3-methyl-*trans*-2-butenylaminopurine (Zeatin)	(0.01; 0.1; 1.0; 2.0) mg	1mg	Higher concentration of Zeatin improving the performance of the culture as well as the development of shoot and root.
7	*trans*-Cinnamic Acid (tCA)	(0.1; 0.5; 1.0) mg	-	In the dark produced etiolated shoots and roots. No significant differences between cultures with tCA and without tCA. However, the number of root and the length of roots were better on culture with tCA kept in the dark.

No.	Substances added to MS media	Amount per liter	NAA per liter	Result
8	Bacto-peptone	2 g	-	Did not give a significant differences to different types of cultures, and insignificant to shoot or root development.
9	Banana	150 g	-	Banana with or without activated charcoal shows a good result on the development of the culture, making the culture firmer.
10	Coconut water	150 cc	-	Did not give a significant differences to different types of cultures, and insignificant to shoot or root development.
11	Activated charcoal	2 g	-	Slightly improved the growth of the culture.

Addition of Zeatin to the media gave the best result for shoot and root development. Different concentrations of Zeatin (0.01; 0.1; 1.0; 2.0 mg/l) showed that higher concentrations of Zeatin improved the performance of the culture, as well as the development of shoot and root. This result confirms the previous trials for axillary buds of *A. titanum* corm using Zeatin (Irawati, 2011).

In this study, cultures with tCA incubated in the dark produced etiolated shoots and roots. There were no significant differences between cultures with tCA and without tCA. However, the number of roots and the length of roots were better on cultures with tCA which were kept in the dark. The effect of etiolation on promoting root development could be applied on cultures without roots. Addition of Benzyl Adenine to culture with tCA should be tried in the future, since it provided good results in media for ginger (Kim and Kim, 1998) and *Phalaenopsis* (Ball *et al.*, 1974-1975)

Banana and coconut water added to the media, either with or without activated charcoal, showed good results on the development of the culture, resulting in a firmer culture. However, further observations should be conducted to confirm this result. The short duration was not enough to lead to a conclusion.

Bacto-peptone was reported as beneficial in recovering vitrified cultures of carnation (*Dianthus caryophyllus* L.) shoots (Sato *et al.*, 1993). In *A. titanum*, addition of 2 g/l Bacto-peptone to the basal medium did not result in significant differences in different types of cultures, and also was insignificant in influencing shoot or root development.

Activated charcoal has strong absorptive properties including of plant growth regulators (auxins or cytokinins) and other substances, but it is commonly used in *in vitro* culture to reduce phenolic inhibitors in the cultures (George, 1996). In *A. titanum* cultures, the effect of activated charcoal slightly improved the growth of the culture.

Histological study Different types of culture were observed and the result of histological study of these cultures are as follows: Figures 5 – 9 Cells and tissues from different types of cultures.

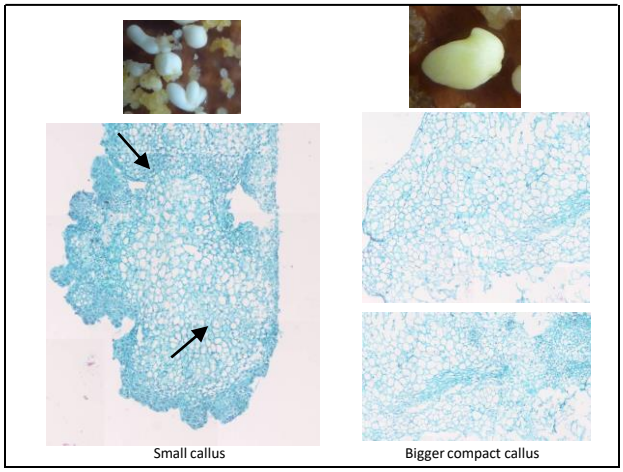

Figure 5. Peripheral cells close to each other (arrow). The shape of the cells are mostly multi-angled, the cells are small with big nucleus. The cells divide in different directions and form unorganized callus (small callus and bigger compact callus).

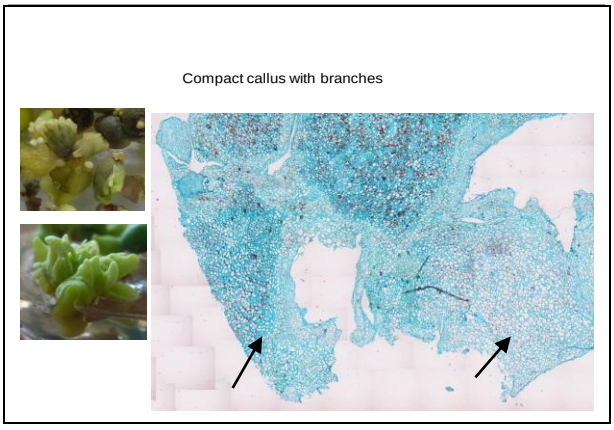

Figure 6. Cells of all branches are similar in their form and their arrangement. The larger cells are rounder and looser (arrow). Some cells are long and quadrangular. The outer part consists of a layer of cells which are close to each other. Some vacuoles containing raphids of oxalic acid crystals (dark blue cells). In some parts the cells are actively dividing.

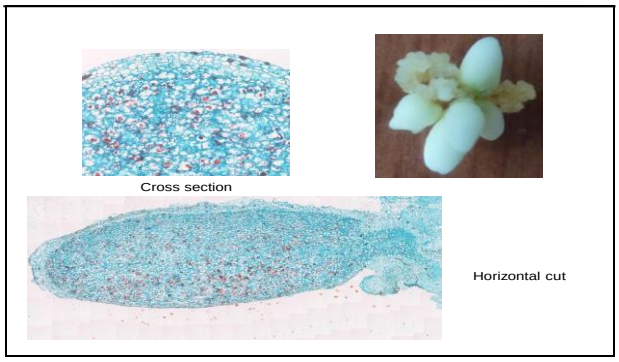

Figure 7. Theree layers of cells, the square-shaped epidermal cells are close to each other, the second and the third layers have bigger cells. The suspensor cells are similar to the upper part. In some part the cells are actively dividing.

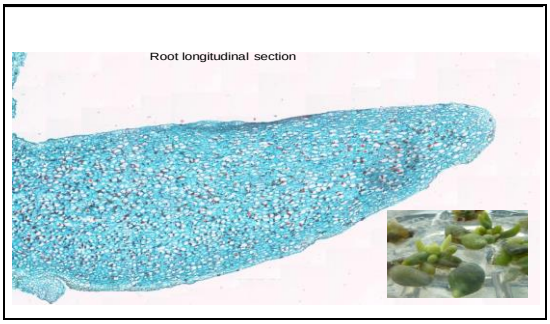

Figure 8. The root-like structures have similar cells to the oval shaped callus

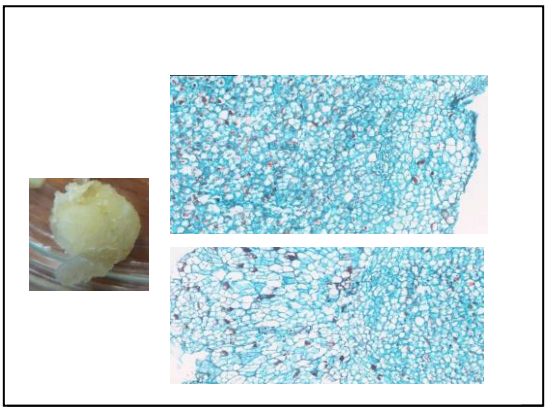

Figure 9. This soft callus has large cells (the nucleus is not clear). The cells are polygonal shaped and loosely arranged.

Histological studies show that the initiation of shoots does not occur in those types of solid cultures. Some cultures shows the cormlike struc-tures, but most of them have irregular shapes. The phenotypic abnormalities often found in cultures after a period of unorganized growth. This seems due to the artificially uncontrolled stimulation of embryogenesis by growth regulators, and the culture loss of regenerative ability, but it can also be due to culture on an inappropriate medium (George, 1996).

Some solid calluses produce more than one plantlet. Normal plantlets usually produce normal roots. In other cultures, only roots appear without shoot, this type of root usually has bigger size and are able to form solid callus when separated from the original culture.

The development of sterile petiole and lamina segments are very good (Fig. 10). This could be used as protocol in propagation of *A. titanum* when the source of plant material is limited as the culture grows well in different media.

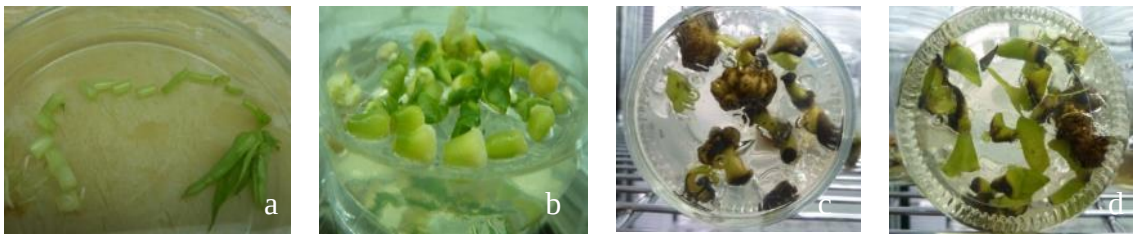

Figure 10. The development of petiole and leaf segments of *Amorphophallus titanum.* a. Original expalnts; b. Development of petiole and leaf segments; c. Compact callus from petiole segment; d. Compact callus from leaf segment.

Acclimatization

When the culture produced shoot(s) and roots, and they were ready to transfer into the shadehouse (Fig. 11). Only cultures produce normal shoots and roots were plant

Figure 11. *Amorphophallus titanum* that acclimatized in 2006 (left) and in 2011 (right).

CONCLUSION

Leaf-veins and leaf-sheaths cataphyll are potential source plant material for propagating *Amorphophallus titanum* and removing sections for propagation do not produce negative effects on the donor plant. The effect of plant growth regulators and organic additives did not give satisfactory results and further tests may alter the conclusions we might draw from this study. Regular sub culturing on fresh medium would improve the development of the culture. When plantlets grow in different types of culture, endogenous hormones also play important roles in the performance of the culture. Hyperhydricity found in many cultures seemed to cause problems in the development of embryos. The histological study shows that the cells of the callus rarely produce organized tissues therefore the shoot development is very limited. The development of cultures originating from sterile material produced better results, and offers the possibility of producing shoots and roots faster.

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